Protocol for BU Neuropathological Assessment

Neuropathological Assessment

Neuropathological evaluation of all autopsied brains is performed by neuropathologists blinded to all demographic and clinical information. Briefly, brains are usually received fresh and the gross neuropathological findings are recorded, including measurement and documentation of all grossly visible vascular lesions and the degree of atherosclerosis in the circle of Willis. The brain with attached brainstem and cerebellum is hemisected with a sagittal cut (half fixed and half to be frozen). Alternate left and right cerebral and cerebellar hemispheres and one half of the brainstem are frozen with the opposing half fixed. The frozen half cerebellar hemisphere is weighed. The cerebral hemisphere to be frozen is cut into 0.5 cm coronal slabs; half of the brainstem is sectioned transversely; half the cerebellum sectioned sagittally. Slabs are then snap frozen at -80 C. The opposite hemisphere is fixed in 4% periodate-lysine-paraformaldehyde (PLP) at 4^0C for at least 2 weeks.

<u>Staining methods</u>: Twenty-one of 41 blocks are cut as 10 µm sections mounted on glass slides and stained with luxol-fast blue, hematoxylin and eosin (LHE), Bielschowsky, and immunostained for AT8, Aß, GFAP, alpha-synuclein, TDP-43 and other antibodies as warranted. The list of regions, histological stains and immunostains for each block is below in Table 1.

Table 1: Key sheet of blocking and staining procedures

Region		LHE	Biel	AT8	Aß-42	α-synucl	TDP-43

#	Region						
1.	Olfactory bulb	X		X		X	
2.	Midbrain at level of red nucleus	X		X		X	X
3.	Precentral, postcentral cortex (BA 4,3,2,1)	X		X			
4.	Inferior parietal cortex (BA 39,40)	X	X	X	X		
5.	Anterior cingulate (BA 24)	X				X	
5A.	Superior frontal gyrus	X		X			
6.	Orbitofrontal gyrus	X		X			
7.	Dorosolateral frontal (BA45, 46)	X	X	X			X
7B.	Prefrontal cortex						
8.	Caudate, putamen, and accumbens (CAP), septal cortex	X			X		
8A.	Anterior insula						
9.	Temporal pole	X		X			
10.	Superior temporal (BA 20, 21,22)	X		X			
11.	Amygdala, with entorhinal cortex (BA 28)	X		X		X	X
12.	Globus pallidus, insula, sub. Innominata	X					
12B.	Insula						
13.	Anterior hippocampus						
14.	Hippocampal formation, lateral geniculate	X	X	X	X		X
15.	Posterior superior temporal gyrus						
16A	Hypothalamus, mammillary body	X		X			
17.	Posterior cingulate gyrus						
18.	Calcarine cortex (BA 17,18)	X		X			

#	Region						
19.	Superior parietal lobule						
20.	Upper pons (level of locus cœruleus)	X		X			
21.	Medulla oblongata with inferior olives)	X		X		X	
22A.	Cervical spinal cord	X		X			
22C.	Lumbar spinal cord (if available)	X		X			
23.	Cerebellar vermis	X		X			
24.	Cerebellum with dentate nucleus	X	X	X	X		
25.	Parastriate cortex						
26.	Cerebellar tonsil						
27.	Pituitary and pineal						
28.	Cerebellum for DNA						
29.	Choroid plexus						

Immunohistochemical procedures: 10 µm sections are cut from paraffin blocks and immunostained. The most commonly used antibodies include: phosphorylated TDP-43 (Cosmo Bio Co., Tokyo, Japan) to detect abnormal TDP-43 inclusions in spinal cord, motor cortex, hippocampus, and cerebellum; phosphorylated tau (AT8, Innogenetics, Inc.) to detect NFT, neuropil threads, Pick bodies, oligodendroglial and glial NFT and ballooned neurons; Aß protein (Dako) to analyze amyloid ß protein deposition as senile plaques, cerebrovascular amyloid, and amorphous deposits; alpha-synuclein (Chemicon) to detect LB, Lewy neurites, pale bodies, or neuronal and glial cytoplasmic inclusions of multiple system atrophy. Additional stains may be performed on a case-by-case basis and may include p62 (Abcam, Cambridge MA) to detect

inclusions in cerebellum and hippocampus; GFAP (Boehringer-Mannheim) to detect astrocytosis; and ubiquitin (Dako) to evaluate LB and inclusions in ALS and FTD. Other antibodies are included as the case warrants. Formic acid pretreatment is used prior to immunostaining for Aß and alpha-synuclein.

Diagnostic Evaluation

Semi-quantitative neuropathological assessment: All cases are scored by Braak and Braak for NFTs (0-VI scale), CERAD scale for plaques (0-3), Thal phase of Aβ plaque accumulation (0-5), cerebral amyloid angiopathy severity and type (0-3, intracortical and leptomeningeal), and Lewy body disease type (olfactory, brainstem, transitional, neocortical, and amygdala-predominant) (1). The presence of TDP-43 inclusions is assessed in multiple regions including spinal cord, medial temporal lobe, medulla, and frontal cortex. For cases with motor neuron disease, the TDP-43 stage is determined using the criteria of Brettschneider et al. (2).

Diagnoses: Neuropathological criteria for the diagnosis of AD/ADRD and other neurodegenerations are used as developed by the NIA-AA Working Group; criteria for CTE follow the recommendations of the NINDS consensus panel (3). The criteria for AD are based on the presence of amyloid-β neuritic plaques and p-tau neurofibrillary tangles according to the NIA–Reagan criteria for intermediate and high likelihood Alzheimer's disease and the recent NIA Alzheimer Association's guidelines (1,4). The NIA–Reagan criteria take into account both the Braak and Braak staging of neurofibrillary tangles (5,6) and the overall density of neuritic plaques based on CERAD criteria (7). The diagnosis of Lewy body disease is based on the presence and distribution of alpha-synuclein-positive Lewy bodies and is considered brainstem-

predominant, limbic or transitional Lewy body disease, and neocortical or diffuse Lewy body disease as defined by McKeith criteria (8) and Braak staging (9,10).

Neuropathological diagnosis of FTLD is based on predominant involvement of the frontal and temporal lobes and characteristic immunohistochemistry for p-tau, TDP-43 and p-TDP-43 using established criteria for FTLD (11-13). The most common FTLD, FTLD with TDP-43-positive inclusions, FTLD-TDP, is defined by TDP-43-positive neuronal cytoplasmic and intranuclear inclusions, dystrophic neurites and glial cytoplasmic inclusions in the superficial layers of cerebral cortex and dentate gyrus. The diagnosis of FTLD-tau, which includes progressive supranuclear palsy, corticobasal degeneration, and Pick's disease, is defined by the specific patterns of p-tau glial and neuronal pathology and neuroanatomical areas of involvement according to consensus criteria (11,14,15).

Consensus criteria for the diagnosis of CTE includes the presence of abnormally phosphorylated tau (ptau) accumulation within neurons, astrocytes, and cell processes in an irregular and patchy distribution that is perivascular and concentrated within the depths of sulci (3,16-18). CTE stage is determined I-IV based on described criteria (18). Other diagnoses may include ALS, which is determined using well characterized pathological criteria (19-21).

Other newer diagnoses not yet incorporated into NACC can be derived from the recorded REDCap variables where available. For instance, a diagnosis of primary age-related tauopathy (PART) can be derived from the Braak NFT and CERAD scores (22), and a diagnosis of limbic-predominate age-related TDP-43 encephalopathy (LATE) can be derived from the presence and location of TDP-43 pathology with or without hippocampal sclerosis (23).

Vascular and Microvascular Lesions

Hippocampal sclerosis is judged by the presence of neuronal loss and gliosis in the hippocampal CA fields and subiculum using the following semi-quantitative scale: 0=none; 1= CA1 only; 2=CA1/Subiculum; 3=Subiculum fields/Prosubiculum; 4= All CA fields, subiculum/Prosubiculum. 'Microinfarcts' are defined as encephalomalacic lesions, 5 mm or smaller in greatest dimension, not identifiable on gross inspection of the brain. They are located in the cortex and subcortical white matter and include cavitated and non-cavitated chronic microinfarcts and microhemorrhages. Cavitated microinfarcts are defined as cystic areas of tissue loss or collapse with gliosis, and usually, macrophage infiltration. Non-cavitated microinfarcts are focal areas of cellular loss and gliosis without the formation of a cystic cavity. Microscopic deposits of blood or hemosiderin with minimal evidence of ischemic infarction are designated as microhemorrhages. Degree of arteriolosclerosis is a composite score of the degree of hyaline thickening of arteriolar walls evaluated semi-quantitatively in deep white matter and basal ganglia. White matter disease is judged using a summary score of myelin loss and cribriform state evaluated in the subcortical white matter and the basal ganglia. Myelin loss is judged by gross inspection of the luxol fast blue, hematoxylin and eosin stained slide and rated semiquantitatively. If the area to be evaluated contains an infarct, the area is not omitted from the analysis. Cerebral amyloid angiopathy is also evaluated in the leptomeninges and in the parenchyma in neocortical regions: middle frontal, inferior parietal, and superior temporal cortices using a scale of severity modified from Von Sattel et al. (24) and Esiri et al. (25). CAA evaluation and scoring are performed as described by Vonsattel et al. on a semi-quantitative 0-3 scale determined by the extent of Aβ deposition within a blood vessel using Aβ

immunohistochemistry [24]. If no Aβ-positive vessels are seen within a given region it is given a score of 0. The presence of Aβ within smooth muscle cells in a normal vessel is classified as mild CAA and given a score of 1. Replacement of the tunica media by Aβ without evidence of hemorrhage is classified as moderate CAA and given a score of 2. Vessel wall deposition of Aβ with evidence of blood leakage or vessel wall fragmentation is defined as severe CAA and given a score of 3 [24]. Leptomeningeal and intracortical vessels are scored separately. A global CAA severity score is determined using NIA-AA guidelines [1]. In brief, no CAA is scored as 0, mild CAA or scattered Aβ-positive vessels = 1; moderate CAA in multiple brain regions = 2; severe CAA in widespread brain regions = 3.

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